BIOETHANOL PRODUCTION FROM CASSAVA BY FERMENTATION PROCESS USING *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Bioethanol can be produced by fermentation of cassava, which carried out in conical flask by using *Saccharomyces Cerevisiae*. Objective of this experiment is to find out the effect of agitation speed, temperature and substrate concentrations on the bioethanol production, cell growth and the glucose consumption. At the end of this experiment, the best substrate concentrations of cassava, which leads to highest production of bioethanol and the highest cell growth can be identified. In this study substrate concentration varied as 25g (6.25%w/v), 50g (12.5% w/v), 75g (18.75% w/v), 100g (25% w/v) and 150g (32.5%w/v). Follows that, the effect of various fermentation temperature will be analyze, by using temperature varies as 20^{o}C, 30^{O}C, 40^{O}C and 50^{o}C. From this temperature vary the growth of yeast and the bioethanol production will be analyzed. Follows that, third parameter of this experiment is agitation speed of fermentation shake flask. Agitation speed varies from 0 rpm, 150rpm, 200 rpm and 250 rpm. For this part, effect of agitation speed on bioethanol production and cell growth of yeast studied. As for the optimum condition, temperature 30^{o}C maintained, agitation speed fixed at 200 rpm, pH maintained at 4.5 and substrate concentration fixed about 100g (25% w/v). This experiment begins with inoculum preparation step, medium preparation step, following by transferring the inoculum into medium into 1L conical flask, fermentation of cassava in incubator shaker and lastly end with sample analysis. From the experiment, agitation speed 200 rpm, substrate concentration of 150g and temperature 40^{O}C gives highest bioethanol production, highest cell growth and highest glucose consumption.

PENGHASILAN BIOETHANOL DARI UBI KAYU MELALUI PROSES PENAPAIAN DENGAN MENGGUNAKAN *SACCHAROMYCES CEREVISIAE*

ABSTRAK

Bioethanol dapat dihasilkan melalui proses penapaian dalam kelalang kon dengan menggunakan *Saccharomyces Cerevisiae*. Tujuan eksperimen ini adalah bagi mengkaji kesan halaju adukan, suhu dan kepekatan substrat ke atas peghasilan bioethanol,pertumbuhan sel yeast dan penggunaan glukosa.Pada akhir eksperiment ini akan diketahui had laju adukan,suhu dan kepekatan substrat yang paling bagus bagi menghasilkan kepekatan etanol yang paling tinggi. Dalam kajian ini kepekatan substrat 25g (6.25% jisim/isipadu), 50g (12.5% jisim/isipadu), 75g (18.75% jisim/isipadu), 100g (25% jisim/isipadu) and 150g (32.5% jisim/isipadu). Tambahan lagi,kesan suhu yang berlainan juga dikenalpasti.antaranya adalah 20^{o}C, 30^{O}C, 40^{O}C dan 50^{o}C. Pada suhu berlainan ini,pertumbuhan sel *Saccharomyces Cerevisiae* dan kepekatan etanol yang dihasilkan akan dikaji.Selain itu, eksperimen diulangi dengan halaju adukan yang berlainan iaitu pada 0 rpm, 150rpm, 200rpm and 250rpm. Suhu penapaian telah ditetapkan pada 30^{o}C dan pH ditetapkan pada 4.5 dan kepekatan substrat akan ditetapkan pada 100g (25%j/i). Eksperimen bermula dengan persediaan baka penapaian ,persediaan medium penapaian dan seterusnya pemindahan baka yang dikulturkan ke dalam medium penapaian ke dam kelalang kon 1L. Penapaian diakhiri dengan analisis sampel.Daripada kajian ini,diketahui bahawa kepekatam substrat 150g, suhu pada 40^{o}C dan halaju adukan pada 200 rpm akan membawa kepada penghasilan etanol yang tinggi dan pertumbuhan sel *Saccharomyces Cerevisiae* yang tinggi.

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LIST OF ABREVIATIONS / SYMBOLS

^{o}C	Degree Celsius
rpm	Round per minute
g	gram
ml	millilitre
CO_2	Carbon Dioxide
%	Percentage
kg	Kilogram
L	Litre
MJ	Milli Joule
w/v	Weight per volume
hr	hour
Et al	And others
g/l	Gram per litre
nm	nanometre
g^{-1}	Per gram
β	beta
v/v	Volume per volume
R^2	Regression
mg/ml	Milligram per millilitre
RI	Refractive Index

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

In recent years, researchers has considered necessary to do structural reforms that allow further development to face the needs of the energy sector. One energy source that is little mentioned in national projects and has demonstrated its feasibility in other regions of the world is the production of ethanol. Cassava (*Manihot esculenta*), sometimes also called manioc, is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes (Leen et al., 2007).Cassava is highly efficient in producing starch and it is tolerant to extreme stress conditions. Furthermore, it fits nicely within traditional farming systems. Fresh roots contain about 30% starch. Cassava starch is one of the best fermentable substances for the production of ethanol.

Fermentation is the oldest way for humans to produce bioethanol, and this is also the traditional way of making alcoholic beverages (Leen et al., 2007). Bioethanol can be produced from biomass by the hydrolysis process and followed by sugar fermentation processes. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin. In order to produce sugars from the cassava, the cassava is pre-treated with acids or enzymes to reduce the size of the feedstock and to open up the plant structure (Saoharit et al., 2009). The cellulose and the hemi cellulose portions are broken down (hydrolysed) by enzymes or dilute acids into sucrose sugar that is then fermented into bioethanol. There are three principle methods of extracting sugars from cassava. These are concentrated acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis (Akihiko et al., 2008).

Previous studies evaluated the environmental impacts of bio based fuels in various categories, including non-renewable energy consumption, greenhouse gas emissions, acidification, eutrophication, human and ecological health, and photochemical oxidation. Most studies have concluded that the use of bioethanol as liquid fuel could reduce greenhouse gas emissions indicated that life-cycle economic, environment and energy assessment provide an important tool for policy makers to better understand trade-offs among economics, environmental impacts and energy for the most effective use of regional energy resources (Hu et al., 2004).

In another study, presented that cassava-based ethanol has a lower net energy, better carbon dioxide emission and lower external cost of carbon dioxide. However, it has higher production cost than conventional gasoline (CG) does, 0.37 MJ/MJ (49% of

CG), 72.61 g/MJ (83% of CG), 0.87 and 0.14 RMB/MJ (200% of CG) respectively. In Guanxi, China the cassava based bioethanol positive net energy and net renewable energy values of 7.475 MJ/L and 7.881 MJ/L, respectively. A study (Nguyen et al., 2007) on the net energy balance and greenhouse gas (GHG) emissions of ethanol from cassava based on a pilot plant data of the Cassava and Starch Technology Research Unit (CSTRU), Kasetsart University, Thailand and found that the energy balance is positive and net avoided GHG emission is 1.6 kg CO2 eq. per litre of ethanol.

On the other hand, in Thailand based on the pilot plant research on cassava starch by CSTRU, Kasetsart University. It was studied that, an energy efficiency (Yu and Toa, 2009) of cassava-based fuel ethanol in Chinese Guangxi by the Monte Carlo method and showed that the energy balance is a positive net energy and energy input to output ratio of 0.7 MJ/MJ. Several LCA studies indicated that in categories of abiotic depletion, GHG emissions, ozone layer depletion, and photochemical oxidation, bioethanol is better fuel than gasoline whereas gasoline is better in terms of human toxicity, ecotoxicity, acidification and eutrophication (Luo et al., 2009). It concluded that cassava-based (Leng et al., 2008) ethanol is energy efficient as indicated by an energy output to input ratio of 1.28 and a major contribution to energy consumption and sulphur dioxide and CO_2 emissions primarily comes from ethanol conversion phase as a result of the combustion of coal to produce energy (Hu et al., 2004b).

1.2 Problem Statement

1.2.1 High Profitability from the Bioethanol Product in Malaysia and Reduce Importation of Bioethanol (Develop Exportation of Bioethanol)

In Malaysia, cassava tubers are used to produce cassava chips and also to produce bioethanol. Now Malaysian government's plan is to have up to 15 ethanol plants in Perak over the next five years (Azlin et al., 2010). This industry working on identifying high productions raw material to produces bioethanol. From the study, it can be assumed that, the production of bioethanol will have a total capacity of 1.22 billion gallons with total profit of 24.4 billion ringgit that is $4.1 billion (Khatijah and Tan, 2000). Hence to improve their profitability towards their production, cassava can be used as the raw material to produce bioethanol. In 2009, Johor agriculture office stated that Malaysia earned 15.3 billion ringgit from the production of cassava chips (Azlin et al., 2010). Hence, it can be conclude that bioethanol industry contributing about 37.3% higher profit compare to cassava chips industry. Investment into bioethanol industry will increase the annual profitability of Malaysian economics.

In addition, development of bioethanol plants may create opportunity to improve local economy. For instance, developing new bioethanol productions area in Malaysia, it can reduce the dependence of bioethanol importation from foreign countries. Malaysia uses bioethanol in pharmaceutical industry (sterilization purposes), production plants (as a reactant to produce polyesters), and in laboratory (for research and development purposes). Hence, by having own production areas Malaysia can reduce toward foreign imports. Moreover, Malaysia also can export the bioethanol to foreign countries such as

Brazil and United States. Those countries mainly depend on the bioethanol as the fuel for the automobile engines (E85 and E20).

There is a current upsurge of interest in the search for renewable biomass(cassava) for the production of transportation fuels like bioethanol, arising especially from the environmental concerns due to the toxic gas emission from petroleum fuels, squeezing petroleum resources and fossil fuels (Shanavas et at., 2010). Bioethanol is the most important biofuel, accounting for more than 90% of the total biofuel use.

Bioethanol can be used in mixtures with fuels for motor vehicles. It can increase the octane index; reducing it between 10 and 15% the CO. Ethanol can be mixed with unleaded gasoline between 10 to 25% without difficulty (Leticia et al., 2010). Ethanol could therefore replace MTBE (methyl-tert-butyl ether), an oxygenated product used in Mexico since 1989, although it has reduced CO2 emissions it has proved to be a groundwater pollutant and has a carcinogenic effect.

Therefore, this research is focused on the production of bioethanol from cassava without pre-treatment by using enzymes. Hence, hydrolysis process does not take place by addition of enzymes but it occurs simultaneously with fermentation.

1.3 Objectives

The objective of this research is to study the bioethanol production from cassava by (*Saccharomyces Cerevisiae)* without enzyme pre-treatment.

1.4 Research Scope

In order to accomplish the objective, 3 scopes were identified:

a) To study the effect of agitation speed on bioethanol production from cassava (*Manihot*) using yeast (*Saccharomyces Cerevisiae).*

b) To study the effect of temperature on bioethanol production from cassava (*Manihot*) using yeast (*Saccharomyces Cerevisiae)*

c) To study the effect of substrate concentration on bioethanol production from cassava (*Manihot*) using yeast (*Saccharomyces Cerevisiae).*

CHAPTER 2

LITERATURE REVIEW

2.1 Cassava

2.1.1 History of Cassava

Cassava (*Manihot esculenta*), sometimes also called manioc, is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes. In Africa, which is the largest centre of cassava production, it is grown on 7.5 million hectare and produces about 60 million tonnes per year. It is a major source of low cost carbohydrates and a staple food for 500 million people in the humid tropics (Leen et al., 2007). The largest cassava market by far is in Nigeria, responsible for 18% of world cassava production. Other important cassava producing countries are Brazil (upcoming), Indonesia, Thailand, Congo and

Mozambique. Approximately 2% of world cassava is traded, mostly in the form of dried chips or pellets (Leen et al., 2007).

2.1.2 Cultivation of Cassava

Major farming activities including land preparing, planting, fertilizing, weeding, and harvesting were covered in this stage (Nguyen et al., 2007). Detailed information on fuel, fertilizers, and herbicides inputs was verified by field survey in the north eastern cultivation area of the country. The total cassava planting area in 2007 was 1.2 million hectare and production yield was 22.9 ton fresh roots per hectare (Pimentel, 1992). When comparing to India which had 0.24 million hectare of cassava planting areas, the production yield was 31.4 ton fresh roots per hectare which was 37% higher than production yield of Thailand (Office of Agricultural Economics, 2008). In traditional agriculture, the most common form of seedbed preparation for cassava planting is on mounts or on unploughed land (Ecoivent, 2006).

On unploughed land, no tillage is done other than required to insert the stem cuttings into the soil. In improved agriculture, the land is first ploughed and then harrowed. Thereafter cassava may be planted on the flat, on ridges or in furrows. Flat plantings of cassava seem to produce higher yields of tuber than ridge or furrow plantings. However, flat planting is unsuitable on heavy clay soils, because the tubers tend to rot. Cassava is propagated vegetatively as clones. Generally, cuttings are taken from the mature parts of the stems, which give a better yield than those taken from the younger portion of the stems (Leen et al., 2007).

Thereafter cassava may be planted on the flat, on ridges or in furrows. Flat plantings of cassava seem to produce higher yields of tuber than ridge or furrow plantings. However, flat planting is unsuitable on heavy clay soils, because the tubers tend to rot. Cassava is propagated vegetatively as clones. Generally, cuttings are taken from the mature parts of the stems, which give a better yield than those taken from the younger portion of the stems. The cuttings should have at least 3 nodes, which serve as origins of shouts and of roots (Leen et al., 2007). Recent releases from agricultural breeding programmes include clones with resistance to many of the major diseases and pests.

Figure 2.1 Cassava plants

Cultivar names are usually based on pigmentation and shape of the leaves, stems and roots. Cultivars may vary in yield, root diameter and length, disease and pest resistance levels, time to harvest, temperature adaptation. Storage root colour is usually

white, but a few clones have yellow-fleshed roots. Each region has its own special clones. Most farmers grow several clones in a field. Cuttings produce root s within a few days and new shoots appear soon afterwards. Early growth is relatively slow, thus weeds must be continuing rolled during the first few months.

Most farmers grow several clones in a field. Cuttings produce root s within a few days and new shoots appear soon afterwards. Early growth is relatively slow, thus weeds must be continuing rolled during the first few months. Although cassava can produce a crop with minimal inputs, optimal yields are recorded from fields with average soil fertility levels (suitable for most food crops) and regular moisture availability. Typically, harvesting can begin eight months after planting. In the tropics, plants can remain unharvested for more than one growing season, allowing the storage roots to enlarge further. However, as the roots age, the central portion becomes woody and inedible (Leen et al., 2007).

2.1.3 Characterization of Cassava

Cassava is a perennial shrub which sometimes reaches the size of a small tree. It stems vary in color from pale to dirty-white to brown marked by numerous nodes formed by scars left by fallen leaves. Pale to dark-green leaves are fan-shape, with 5 to 9 lobes. Roots of cassava plants are few and swallow and some become storage roots. These are clustered around the base of the plant and extend about 60 cm on all sides (Balinghoy, 2009). It is for these roots which contain from 15 to 40 percent starch that

the crop is cultivated. Under favorable conditions, a single root may weigh as much as four kilos.

The number of roots per plant at harvest varies from 2 to 7 each averaging 27.7 to 43.3 cm long and from 4.5 to 7.4 cm in diameter. Cassava is a tropical and sub-tropical plant. It grows in regions with more or less evenly distributed rainfall through out the year. An ambient temperature that ranges from 25°C -30°C. Select an open field with sandy loam or clay loam soil. Be sure that the area is not prone to waterlogging; it must be a well-drained soil (Kamoteng, 2009). Also consider the soil fertility with pH range of 5.5-6.5. Cassava thrives at sea level to 845 meters above sea level. It grows best when planted at the start of the rainy season. Figure 2.2 shows the cassava tubers.

Figure 2.2 Cassava Tuber

2.1.4 Applications of Cassava

Cassava is an important food crop for developing countries, being the main source of energy for between 200 and 300 million people (Gevaudan and Didierm, 1989). In Tanzania, cassava is an important subsistence food crop, although it is still considered by many people outside the production areas as a famine reserve crop when cereals, especially maize, fail. Around 84% of total production in the country is utilized as human food. The remaining fraction is used for livestock feed, starch making and export. This crop is bulky and highly perishable, but is available all year round thus contributing to food security. Its high energy content helps in minimizing incidences of energy malnutrition (Hahn et al., 1970). Cassava also used to produce bioethanol (Akihiko et al., 2008).

Such flours could be blended with cereal flours to improve acceptability of the cassava-based porridges. Exact proportions of these blends have not been fully established. Cassava has also been used in baked products and fried products like doughnuts, buns and *chapati* (a pan-fried unleavened flat round wheat-based product), although not to the extent of the stiff porridges. Another area of utilization of cassava is in the starch industry for food and non-food uses. This product can be obtained from the fresh dried cassava (Hahn et al., 1970). The easiest form of extraction of this cassava starch is from the fresh cassava using graters to grate the cassava into a fine paste.

Yake yake is one paste product obtained after peeling, washing, grating, drying and sieving the cassava to obtain a meal that is moulded and steam-baked. *Agbeli kaklo*

is a second product encountered in literature resembling *yake yake* but instead of steam-baking, the meal is mixed with meat, using the hand it is moulded into small cylinders and palm kernel or coconut oil is used for frying (Doku, 1969**)**. Flour is one of the most important cassava products. Steamed paste and wet paste are common in other places but not Tanzania, and so are toasted and steamed granules (Hweke, 1994**)**.

2.2 *Saccharomyces Cerevisiae*

2.2.1 History of *Saccharomyces Cerevisiae*

Saccharomyces Cerevisiae which in Latin means "sugar fungus" has been utilized by human for thousands of years. It is believed that it was first discovered on the skins of grapes (Polsinelli, 1999). *Saccharomyces Cerevisiae* is budding or brewing yeast, and has been put to use since antiquity to make dough rise and to provide ethanol in alcoholic beverages. The natural history of *Saccharomyces Cerevisiae* has been obscured in part by a long history of domestication. It is the microbial agent responsible for the fermentation of wine, beer and other alcoholic beverages, and the most commonly used microbial leavening agent for bread. Cavalieri has identified *Saccharomyces Cerevisiae* in the residue inside an Egyptian wine jar from c. 3150 B.C (Cavalieri, 2003).

The natural strains of *Saccharomyces Cerevisiae* described in the literature have generally been isolated from vineyard grapes and other fruits (Polsinelli, 1999), fermentation facilities (Mortimer, 1994), insects, oak fluxes (Naumov et al., 1998) or